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ORIGINAL ARTICLE

Association between serum resistin level and outcomes in kidney transplant recipients

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Summary

Resistin is an adipocytokine that is associated with inflammation, coronary artery disease, and other types of cardiovascular disease among patients with normal kidney function. However, little is known about the association of resistin with outcomes in kidney transplant recipients. We collected socio-demographic and clinical parameters, medical and transplant history, and laboratory data from 988 prevalent kidney transplant recipients enrolled in the Malnutrition-Inflammation in Transplant—Hungary Study (MINIT-HU study). Serum resistin levels were measured at baseline. Associations between serum resistin level and death with a functioning graft over a 6-year follow-up period were examined in unadjusted and adjusted models. The mean \pm SD age of the study population was 51 \pm 13 years, among whom 57% were men and 21% were diabetics. Median serum resistin concentrations were significantly higher in patients who died with a functioning graft as compared to those who did not die during the follow-up period (median [IQR]: 22[15–26] vs. 19[14–22] ng/ml, respectively; $P < 0.001$). Higher serum resistin level was associated with higher mortality risk in both unadjusted and fully adjusted models: HRs (95% CI): 1.33(1.16–1.54) and 1.21(1.01–1.46), respectively. In prevalent kidney transplant recipients, serum resistin was an independent predictor of death with a functioning graft.

Introduction

Resistin is an adipose tissue-specific hormone, which was discovered in the past decade by three independent research groups [1]. It is a 12.5-kDa cysteine-rich protein that has different isoforms circulating in the bloodstream [2–4]. Resistin is linked to insulin resistance, hence the name.

Resistin also appears to play an important role in the immune system. It is largely synthesized in macrophage cells; induces the production of inflammatory cytokines; elevates the expression of cell adhesion molecules, such as

vascular cell adhesion molecule (VCAM), intracellular cell adhesion molecule (ICAM), and monocyte chemotactic protein (MCP); and promotes vascular smooth muscle proliferation and monocyte vascular infiltration [5–7].

Resistin may influence patient outcomes via multiple diverse biological pathways. Although controversial, human data suggest that resistin is linked to insulin resistance and obesity [8–10]. Other studies have found that resistin functions as a pro-inflammatory cytokine and its serum level was shown to correlate with serum tumor necrosis factor-alpha (TNF-alpha) and interleukin 6 (IL-6)

levels [11]. Higher resistin levels have also been associated with chronic inflammatory diseases such as rheumatoid arthritis or inflammatory bowel disease, and may play a role in the pathophysiology of atherosclerosis and endothelial cell injury [12,13]. Based on these data, resistin may be a biomarker and potentially a pathophysiological factor in the development of cardiovascular disease and death [14–17].

While there have been a number of prior studies examining resistin in patients with chronic kidney disease (CKD), only one study to date has been performed among kidney transplant recipients [18]. In this study, higher serum resistin was associated with markers of chronic inflammation (e.g., high-sensitive C-reactive protein [CRP] and white blood cell count) as well as impaired kidney function. Based on these results, resistin may be a risk factor for mortality in patients with CKD. To further inform the field, our primary aim was to analyze the association between serum resistin levels and outcomes in prevalent kidney transplant recipients. We hypothesized that higher resistin levels were associated with higher risk of death and graft loss.

Methods

Study population and data collection

We recruited all prevalent kidney transplant recipients ($n = 1214$), who were followed at a single transplant outpatient clinic at the Department of Transplantation and Surgery at Semmelweis University Faculty of Medicine in Budapest, Hungary, during the inclusion period of December 31, 2006 to December 31, 2007 (Malnutrition-Inflammation in Transplant—Hungary Study [MINIT-HU Study]) [19–25]. Baseline characteristics were defined as data detected at the time of cohort inclusion. We excluded patients who at the time of study entry experienced acute rejection within the last 4 weeks, were hospitalized at the study entry, received kidney transplantation in the previous 3 months, or had acute infection or bleeding. The study cohort algorithm is shown in Figure S1 and resulted in 988 patients.

Medical history and socio-demographic data were collected at baseline, including information on age, sex, etiology of chronic kidney disease, co-morbidities (the modified Charlson Comorbidity Index (CCI)), and transplantation-related data including immunosuppressant medications [26]. Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [27].

The study was approved by the Ethics Committee of Semmelweis University (49/2006). Before enrollment, patients received detailed written and verbal information

regarding the aims and protocol of the study and gave written consent to participate.

Laboratory data

All laboratory data were collected and measured at the baseline clinic visit and included resistin, TNF-alpha, IL-6, blood hemoglobin (Hb), serum CRP, serum creatinine, blood urea nitrogen (BUN), and serum albumin levels. Serum resistin concentration was measured using immunoassay kits based on solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN, Coefficient of Variation <10%).

Kidney transplantation-related data and donor characteristics

Transplant-related data were obtained from the medical records and included medications (including current immunosuppressive treatment), transplant vintage (i.e., time elapsed since the date of transplantation), length of time on dialysis prior to transplantation, type of allograft, history of acute rejection(s) that were treated after transplantation, human leukocyte antigen (HLA) mismatch, panel-reactive antibodies titer (PRA), cold ischemia time (CIT), donor age and sex, and history of delayed graft function. Total time with end-stage renal disease (ESRD) was defined as the total time on any type of renal replacement therapy including any type of dialysis or kidney transplantation.

Immunosuppressive therapy

Standard immunosuppressive therapy included prednisolone, with either cyclosporine (CsA) A microemulsion formulation (Neoral) or tacrolimus, combined with mycophenolate mofetil (MMF) or azathioprine or sirolimus.

Outcome ascertainment

Patients were followed for a median (IQR) period of 76 (46–79) months. The primary outcome of interest was all-cause death with a functioning graft. We also assessed the association between baseline serum resistin level and death-censored graft loss as a secondary outcome. Deaths and re-initiations of maintenance dialysis were ascertained from hospital medical records. Deaths were validated by cross-referencing with the Hungarian Central Office of Administrative and Electronic Public Service record, which is the government agency maintaining official vital status data.

Statistical analysis

Statistical analyses were carried out using STATA 13 (Stata-Corp, College Station, TX, USA) software. Descriptive data were summarized using proportions, means (\pm standard deviation, SD), or medians [IQR] as appropriate. Categorical variables were compared using chi-square tests, and continuous variables were compared using Student's *t*-test or the Mann–Whitney *U*-test as appropriate and *P* for trend test. Correlations between covariates were assessed by Pearson correlation coefficients. In all analyses, two-sided tests were used and the results were considered statistically significant if the *P*-value was <0.05 .

The association between baseline serum resistin level and deaths with a functioning graft was assessed using Cox-proportional regression analysis and Kaplan–Meier plots with the log rank test. Analogous analyses were also conducted for death-censored graft loss as a secondary outcome. Proportional hazards assumptions were tested using scaled Schoenfeld residuals. The variables entered in the multivariable-adjusted models were selected based on theoretical considerations; we included predictors in the models which were known to be associated both with resistin levels and with mortality based on scientific evidence, and which were available in our database. Five Cox regression models were examined with incremental levels of multivariable adjustment: (i) unadjusted model; (ii) model 1 was adjusted for age and sex; (iii) model 2 was adjusted for variables in model 1, as well as baseline eGFR, CCI, ESRD time, and diuretic treatment at baseline; (iv) model 3 was adjusted for variables in model 2, as well as serum albumin level and body mass index (BMI); (v) model 4 was adjusted for variables in model 3 and cold ischemia time, PRA level, HLA mismatch, number of transplantations, TNF-alpha, IL-6, and CRP levels.

In sensitivity analyses, we also assessed the association between baseline resistin levels and all-cause mortality. Because death with functioning graft and graft loss are competing events, a competing risk model was used to better analyze the risk of death with a functioning graft using the Fine and Gray model [28]. Our event of interest was death with functioning graft, and the competing event was graft failure. We also performed subgroup analyses in clinically relevant subgroups of patients, and the *P*-values of the interaction terms were calculated.

Only 6% of the data were missing in our final model; therefore, we had all the variables from 933 patients (Table S6). Missing values were not imputed in primary analyses, but were substituted in our sensitivity analyses with the use of multiple imputation procedures (creating 5 datasets) using STATA's "mi" set of command in sensitivity analyses (Table S3). We followed the STROBE guidelines in our article (Table S7).

Results

Demographics and baseline characteristics

Baseline characteristics are shown in Table 1. The mean age \pm SD of the population was 51 ± 13 years, 57% were male, 21% had diabetes mellitus, 9% had coronary heart disease, and the median time since kidney transplantation was 72 months.

Patients in the highest resistin tertile had significantly lower residual graft function, higher levels of inflammatory markers, lower BMI and triglyceride levels and spent more time on any type of renal replacement therapy (i.e., longer ESRD time) than patients in the lower resistin tertiles (Table 1).

Serum resistin levels showed strong negative correlation with eGFR (Fig. 1); weaker negative correlations with BMI, abdominal circumference, and triglyceride levels; and had positive correlations with adiponectin, inflammatory, and demographic parameters as shown in Table S1.

Mortality

Figure 2 shows the association between higher serum resistin level in 10 ng/ml increments and outcomes. Serum resistin levels at baseline were significantly higher in patients who died with a functioning graft as compared to patients who were alive with functioning graft at the end of the study period (median[IQR]: 22[15–26] and 19[14–22] ng/ml, respectively). Patients in the lower serum resistin tertile had early separation in their survival curves from their counterparts in the higher serum resistin tertiles (Fig. 3 panel a). There were 182 deaths over a median follow-up period of 76 months; the rate of death with a functioning graft was 36/1000 patient-years (95%CI: 31–42). Crude mortality rates by resistin tertiles and outcomes are presented in Table S2.

Figure 4 panel A shows a strong linear positive association of serum resistin as a continuous variable and risk of death using fractional polynomials and cubic splines. This analysis has revealed that each 10 ng/ml higher serum resistin level was associated with 33% higher risk of mortality (HR [95%CI]: 1.33 [1.16–1.54]), and this strong association remained qualitatively the same even after adjustment for confounders in our fully adjusted model: HR (95%CI): 1.21 (1.01–1.46) (Fig. 2, Table S3).

Compared to patients in the lowest serum resistin tertile, those in the middle tertile had similar mortality risk (HR [95%CI]: 1.01 [0.68–1.49]), while patients who were in the highest tertile showed a trend toward higher mortality risk in the multivariable-adjusted model: HR (95%CI): 1.22 (0.82–1.84) (Table S4). We also assessed the association between serum resistin level in 10 ng/ml increments and mortality risk in various subgroups of patients using multivariable-adjusted Cox regression anal-

Table 1. Baseline characteristics of the 988 kidney transplant recipients.

	All patients (n = 988)	First tertile of serum resistin (n = 330)	Second tertile of serum resistin (n = 329)	Third tertile of serum resistin (n = 329)	*P value
Resistin (ng/ml)	20.60 ± 9.03	12.68 ± 2.47	18.66 ± 1.80	30.48 ± 8.43	<0.001
Demographic parameters					
Graft loss (%)	20	12	17	32	<0.001
Mortality (%)	18	15	18	23	<0.001
Age (year)	51 ± 13	52 ± 12	51 ± 13	50 ± 13	0.038
Sex—Male (%)	57	61	56	55	0.324
Time since Tx (month)	72 (114-39)	64 (110-36)	68 (111-37)	83 (119-47)	<0.001
Previous time on dialysis (month)	20 (38-9)	18 (36-8)	22 (38-10)	22 (42-8)	0.041
Total ESRD time (month)	108 (154-68)	96 (147-63)	103 (152-64)	118 (162-84)	<0.001
Number of Tx.(%)					
1	88	90	88	86	0.111
2	10	9	10	13	
3	1	1	2	1	
Charlson comorbidity index	2 (4-2)	2 (4-2)	2 (4-2)	2 (3-1)	0.937
Presence of HT (%)	94	94	93	94	0.698
Presence of DM (%)	21	22	22	19	0.552
Presence of coronary heart disease (%)	9	9	8	10	0.609
Smoking (%)	19	21	16	20	0.242
Kidney function-related parameters					
Hgb (g/l)	134.7 ± 17.0	138.6 ± 14.8	135.2 ± 16.6	130.4 ± 18.4	<0.001
eGFR (CKD-EPI) (ml/min/1.73 m ²)	50.9 ± 21.0	61.3 ± 18.2	51.4 ± 19.2	39.8 ± 19.7	<0.001
Serum albumin (g/l)	40.2 ± 4.1	40.8 ± 3.5	40.4 ± 4.2	39.5 ± 4.6	<0.001
Creatinine (umol/l)	144.8 ± 82.5	113.3 ± 38.4	134.5 ± 50.6	186.7 ± 116.6	<0.001
HCT (%)	38.4 ± 4.7	39.2 ± 4.1	38.6 ± 4.6	37.5 ± 5.1	<0.001
ESA treatment (%)	10	6	8	17	<0.001
Inflammation					
WBC (10 ⁹ /l)	7.9 ± 2.3	7.2 ± 2.0	7.9 ± 2.2	8.6 ± 2.5	<0.001
CRP (mg/l)	3.1 (6.8-1.5)	2.6 (5.3-1.4)	3.1 (7.1-1.4)	3.9 (8.3-1.6)	<0.001
IL6 (pg/ml)	3.1 (3.6-1.5)	1.9 (3.9)	3.0 (3.6-1.3)	3.6 (4.3-1.4)	0.001
TNF-alpha (pg/ml)	2.1 (2.8-1.5)	1.8 (2.3-1.3)	2.2 (2.9-1.6)	2.3 (3.2-1.7)	<0.001
Nutrition					
BMI (kg/m ²)	27.0 ± 4.9	27.5 ± 4.9	27.0 ± 4.9	26.5 ± 4.9	0.002
Abdominal circumference (cm)	99 ± 14	100 ± 13	98 ± 15	98 ± 15	0.036
HDL (mmol/l)	1.3 ± 0.4	1.4 ± 0.5	1.3 ± 0.4	1.2 ± 0.4	<0.001
LDL (mmol/l)	3.2 ± 1.0	3.3 ± 0.9	3.2 ± 0.9	3.0 ± 1.0	<0.001
Cholesterine (mmol/l)	5.5 ± 1.3	5.7 ± 1.2	5.5 ± 1.2	5.3 ± 1.8	<0.001
Triglyceride (mmol/l)	1.7 (2.5-1.2)	1.7 (2.5-1.2)	1.7 (2.5-1.2)	1.7 (2.5-1.2)	0.904
Transplantation-related data					
Primary cause of ESRD (%)					
Chronic GN	23	22	22	24	0.235
Chronic TIN	13	12	12	16	
PKD	18	21	18	16	
Diabetic nephropathy	5	5	6	3	
Hypertensive nephropathy	7	5	8	7	
Others or unknown	23	20	24	24	
Cold ischemic time (min)	1249 ± 347	1218 ± 354	1249 ± 355	1281 ± 330	0.008
History of delayed graft function (%)	26	22	29	27	0.151
PRA mean (min, max)	3.80 (0–85)	2.10 (0–60)	4.20 (0–85)	5.10 (0–70)	0.015

Table 1. continued

	All patients (n = 988)	First tertile of serum resistin (n = 330)	Second tertile of serum resistin (n = 329)	Third tertile of serum resistin (n = 329)	*P value
HLA mismatch (%)					
0	1	1	1	1	0.438
1	5	6	5	4	
2	22	20	20	25	
3	46	48	44	46	
4	21	20	24	20	
5	4	4	4	4	
6	1	2	2	0	
Immunosuppression					
Steroid use (%)	81	77	82	85	0.027
Cyclosporine use (%)	49	46	49	51	0.347
Tacrolimus use (%)	43	43	43	43	0.992
Azathioprine use (%)	4	4	6	2	0.077
MMF use (%)	78	76	79	79	0.664
Sirolimus use (%)	8	12	8	4	0.001
Everolimus use (%)	2	3	2	2	0.549

HT, hypertension; DM, diabetes mellitus; ESRD, end-stage renal disease; Hgb, hemoglobin; eGFR, estimated GFR; CKD-EPI, Chronic Kidney Disease – Epidemiology Collaboration; ESA, erythropoietin-stimulating agent; HCT, hematocrit; WBC, white blood cell count; CRP, C-reactive protein; IL6, interleukin-6; TNF-alpha, tumor necrosis factor-alpha; BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; GN, glomerulonephritis; TIN, tubulo-interstitial nephritis; PKD, polycystic kidney disease; PRA, platelet-reactive antigen; HLA, human leukocyte antigen; MMF, mycophenolate mofetil; number of Tx: number of transplantations. Values are in median with interquartile range or in mean±SD. The bold values are the mean±standard deviation (SD) of the serum resistin level.

*P value comparing the resistin subgroups.

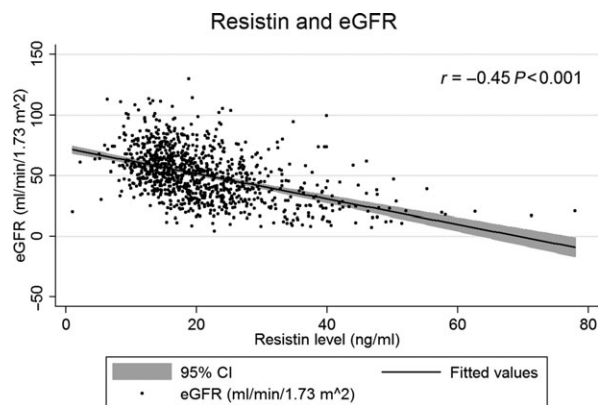


Figure 1 Association between serum resistin level and estimated glomerular filtration rate (eGFR).

yses (Figure S2 panel A). Similar associations between increased serum resistin levels and higher mortality risk were detected in almost all subgroups, although tests of interactions were statistically significant in some subgroups indicating effect modification by age, BMI, and serum albumin levels.

In sensitivity analysis, we assessed the association between serum resistin levels and all-cause mortality. Qualitatively similar associations were found using all-cause mortality as an alternative outcome (Figs 2, 3 panel b, S2

panel b, and Tables S2–S4). Similar results were found after multiple imputations (Table S3). Similar trends were found in our competing risk regression models (Table S5).

Graft loss

Patients in the lowest serum resistin tertile had early separation in the graft survival curve from their counterparts in higher serum resistin tertiles (Fig. 3 panel c). There were 201 graft losses, and the event rate was 40/1000 patient-years (95%CI: 35–46), which positively correlated with higher serum resistin level (Table S2).

Figure 4 panel C shows a strong linear and positive association between serum resistin level and risk of graft loss using fractional polynomials and cubic splines. Each 10 ng/ml higher serum resistin level was associated with a 71% higher risk of graft loss (HR [95%CI]: 1.71 [1.54–1.89]), and this association remained qualitatively the same even after adjustment for confounders in our fully adjusted model: HR (95%CI): 1.71 (1.49–1.96) (Fig. 2, Table S3).

Compared to patients in the lowest serum resistin tertile, patients in the middle tertile experienced a 53% higher risk of graft loss (HR [95%CI]: 1.53 [0.99–2.36]) while those in the highest tertile had a threefold higher risk: HR (95%CI) 3.06 (2.03–4.60) in multivariable-adjusted models

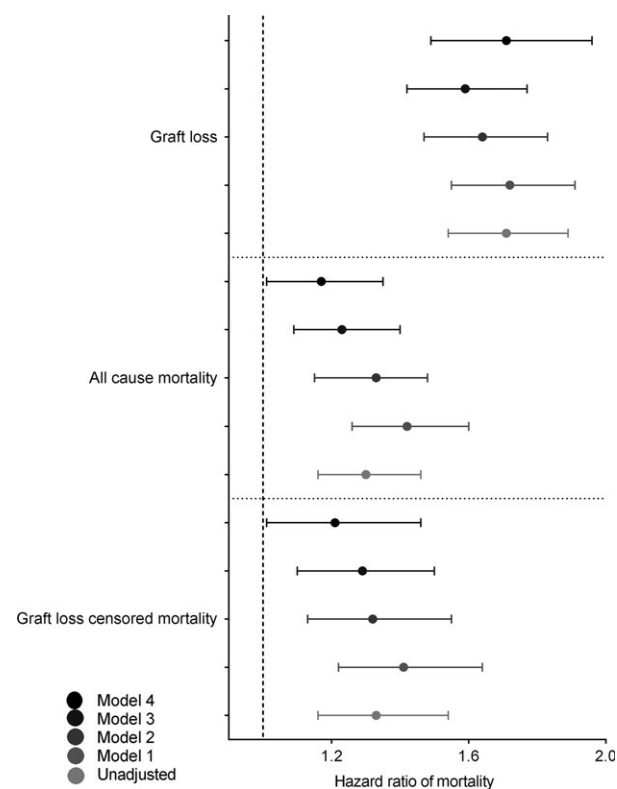


Figure 2 Association between the resistin level in 10 ng/ml increments and outcomes using Cox-proportional models and logistic regression models in 988 kidney transplant recipients. Data were adjusted for the following: model 1: age, sex; model 2: model 1 covariates and baseline eGFR, CCI, ESRD time, diuretic treatment; model 3: model 2 and albumin level, BMI; model 4: model 3 and cold ischemic time, PRA level, HLA mismatch, number of transplantations, TNF- α , IL-6, and CRP. Abbreviations: eGFR: estimated glomerular filtration rate; ESRD time: total time spent on any type of renal replacement therapy; CCI: Charlson Comorbidity Index, BMI: body mass index; PRA: panel-reactive antibodies titer; HLA: human leukocyte antigen; TNF- α : tumor necrosis factor- α ; IL-6: interleukin 6; CRP: C-reactive protein.

(Table S4). Similar results were found after multiple imputations (Table S3). Similar associations between higher serum resistin levels and higher risk of graft loss were detected in almost all subgroups (Figure S2 panel C).

Discussion

To the best of our knowledge, this is the first observational cohort study demonstrating that higher serum resistin levels are independently associated with adverse outcomes in a large cohort of prevalent kidney transplant recipients. We hypothesized that higher resistin levels were associated with higher risk of mortality and graft loss. In our prevalent cohort, higher serum resistin level was associated with higher mortality risk in both unadjusted and fully adjusted models. Each 10 ng/ml elevation of serum resistin was

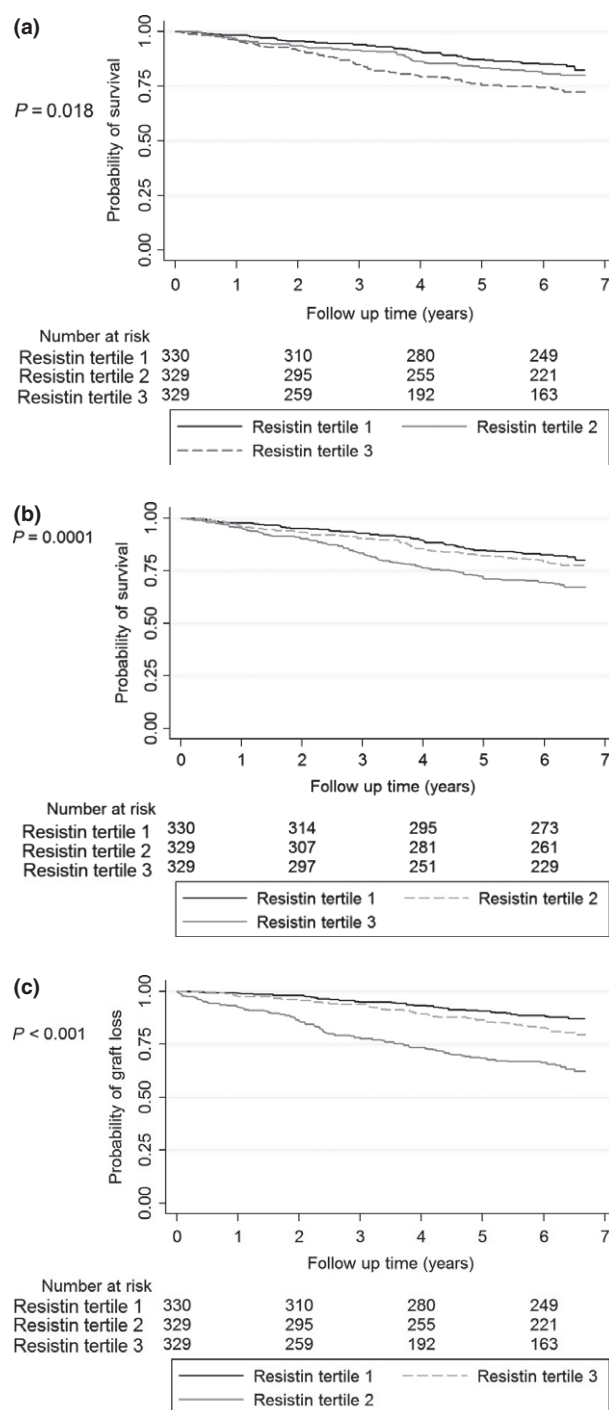


Figure 3 Kaplan-Meier curves showing the association between serum resistin tertiles and death with a functioning graft (panel a), all-cause mortality (panel b), and death-censored graft loss (panel c) for 988 kidney transplant recipients.

associated with 33% higher risk of mortality in unadjusted models, and 21% higher risk of mortality after adjustments.

Serum resistin levels showed strong negative correlations with residual graft function and weakly positive association

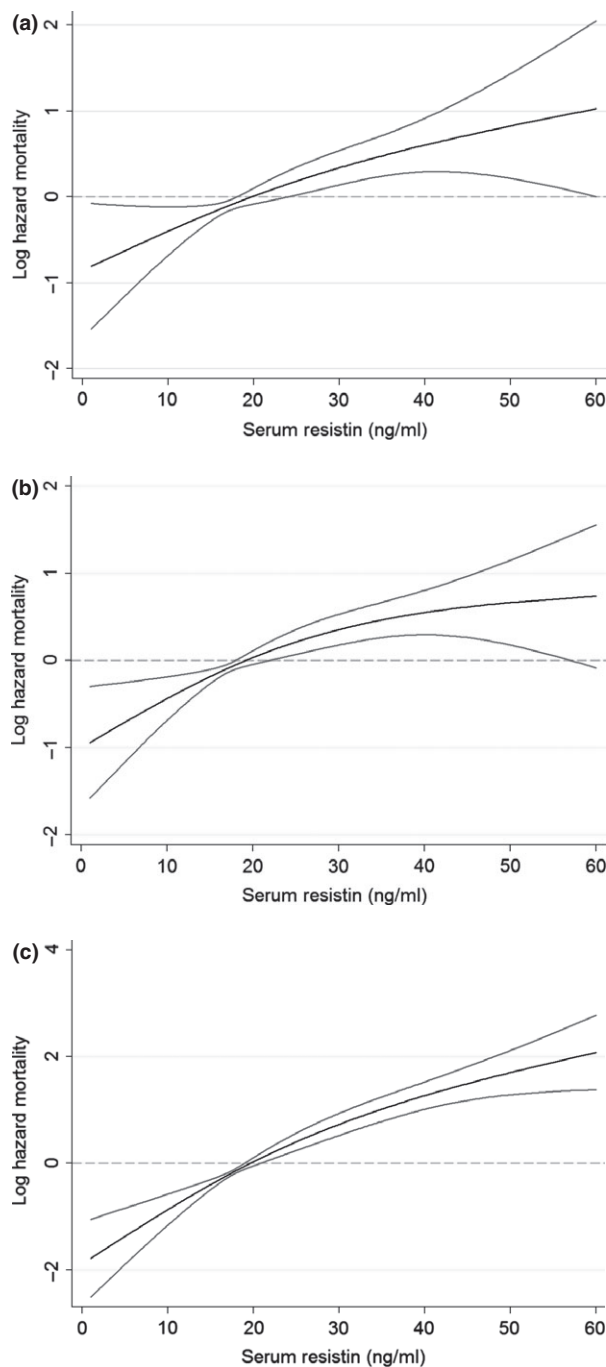


Figure 4 Association of resistin levels with death with functioning graft (panel a), all-cause mortality (panel b), and death-censored graft loss (panel c) in unadjusted Cox regression models in 988 kidney transplant recipients.

with inflammatory markers, while no association was detected between serum resistin and body weight or presence of diabetes. There are two potential explanations for elevated serum resistin levels in the kidney transplant recipient population, which include (i) impaired renal excretion

of resistin, and (ii) increased synthesis of resistin caused by cytokines. Similar associations between serum resistin, kidney function, and inflammation have been observed in previous studies [29]. Axelsson *et al.* [30] examined the role of resistin in 239 individuals with CKD and found strong associations of resistin with GFR and inflammatory markers. Malyszko *et al.* [29] demonstrated that patients on hemodialysis with residual kidney function have significantly lower resistin levels than those without residual renal function, supporting the hypothesis that resistin is excreted via the kidney. To the best of our knowledge, only one study has assessed these associations in kidney transplant recipients [18]. Among 80 transplant recipients, Malyszko *et al.* [18] found that resistin was associated with both eGFR and inflammatory markers such as CRP and IL-6. According to Marouga *et al.* [31], resistin could be the mechanistic link between the protein energy wasting syndrome and inflammation, in which patients with lower BMI have higher levels of inflammatory factors as well as resistin levels. In our study, we found only weak associations with these inflammatory markers, while the association with residual renal function was more prominent.

There was also a strong association between higher serum resistin level and higher risk of death in our cohort. Each 10 ng/ml higher serum resistin was associated with a 17% higher risk of death even after adjusting for confounders such as socio-demographic parameters, residual kidney function, nutritional/inflammatory parameters, comorbidities, and transplantation-related covariates. Our findings are similar to what was recently described across various types of heterogeneous populations in a meta-analysis, although not all studies detected a resistin–mortality association [32,33]. There are several different pathways that may explain the association between resistin and death. First, increased serum resistin may lead to death via cardiovascular processes. In a prospective study with 6 years of follow-up, Frankel *et al.* [34] found that higher levels of resistin were associated with a higher risk of incident heart failure. Similar results were found in a prospective study by Weikert *et al.* [15], who observed that patients in the highest resistin quartile had two times higher risk of myocardial infarction compared to patients in the lowest quartile. Several studies have investigated the association of resistin with mortality in patients with impaired kidney function, although none have been conducted in kidney transplant populations. Spoto *et al.* [35] investigated the association of resistin with both all-cause and cardiovascular mortality in 231 patients with ESRD. Strong associations were observed between serum resistin and mortality, although serum adiponectin was a strong effect modifier of this relationship. In contrast, Chung *et al.* [36] reported that the lowest serum resistin level was associated with the highest rate of hospitalization among hemodialysis patients. In

animal studies, Zhang *et al.* [37] found that higher resistin levels caused myocardial dysfunction and that the differential molecular and vascular effects of resistin could lead to coronary heart disease, kidney damage, as well as graft loss. Further studies are needed to determine the mechanisms underlying the association between serum resistin, cardiovascular disease, and death.

Another novel finding in our study was the association between increased serum resistin level and higher risk of graft loss in our population. While a number of studies have examined the association between resistin and kidney function, there have been no prior studies investigating the association of resistin with graft loss [38]. As previously mentioned, serum resistin levels show strong correlations with inflammatory markers and may possibly play an important role in endothelial function. Resistin itself is enhancing the synthesis of inflammatory cytokines in macrophages and in *in vitro* experiments. Resistin has been shown to be associated with higher expression of cell adhesion molecules (VCAM, ICAM, MCP) [5,7]. In addition, Calabro *et al.* [6] has found that increased resistin levels have a dose-dependent association with smooth muscle proliferation and monocyte vascular infiltration in human aortic smooth muscle cells. In addition, Verma *et al.* [39] discovered that resistin stimulating the release of endothelin-1 and nitrogen monoxide in endothelial cells. The association between resistin and endothelin-1 was also examined in a clinical study. [40] All these vascular and inflammatory effects could lead to endothelial dysfunction, glomerulo-sclerosis, and tubulo-interstitial fibrosis resulting in higher risk of graft loss.

The possible clinical relevance of our findings in kidney transplant recipients is not immediately clear at this point. Recently, *in vitro* studies have suggested that statin use could lower resistin and TNF-alpha levels preventing graft loss or cardiovascular disease; however, a recent clinical study did not support this hypothesis [41,42]. On the other hand, statins such as fluvastatin, which decrease resistin levels, have shown a positive impact on kidney transplant population outcomes [7,43,44]. The other promising group of medications includes thiazolidinediones, which have been shown to inhibit resistin synthesis *in vitro* [45]. However, it has yet to be shown that lowering resistin levels with medications can directly improve outcomes in the kidney transplant population.

The strength of our study includes its examination of a large cohort of kidney transplant recipients, a relatively long period of follow-up time, and minimal missing data given the protocolized study design and data collection. In addition, our analyses accounted for important confounders of the resistin–mortality association such as residual graft function and inflammatory markers. To the best of our knowledge, our study is the first to investigate the

association of serum resistin with mortality and graft loss in prevalent kidney transplant recipients.

The results of our study should be tempered by some potential limitations. One of the major limitations of this study was that serum resistin level was measured only once at baseline; therefore, we did not have the ability to follow change in serum resistin levels over time and perform time-dependent analysis. Furthermore, we do not have serum resistin measurement performed prior to kidney transplantation, so we were not able to adjust for these values in our models. Moreover, a prevalent cohort has been used to assess the association between serum resistin and outcomes, which could have introduced selection bias. In addition, we do not have information about proteinuria, which could be an important confounder. Additionally, models could only be adjusted for identified confounders for which we had available data. Therefore, we cannot rule out residual confounding. Due to data limitations, we could not analyze associations of serum resistin with cause-specific mortality.

Conclusions

In our relatively large and contemporary cohort of almost one-thousand kidney transplant recipients, we found that serum resistin showed moderate-to-strong associations with residual graft function and weak correlations with inflammatory markers. In addition, we reported strong, linear associations between serum resistin level and clinical outcomes such as mortality and graft loss. Further studies are needed to examine whether treatments decreasing serum resistin can favorably impact outcomes in kidney transplant recipients.

Authorship

KN: contributed to analysis of the data, interpretation of data, and wrote the manuscript. AU: contributed to data collection and writing the manuscript. MEC: contributed to data collection and writing the manuscript. AR: contributed to data collection and writing the manuscript. CPK: contributed to interpretation of data and writing the manuscript. ZM: contributed to writing the manuscript. CMR: contributed to writing the manuscript. IM: contributed to data collection, contributed to interpretation of data, and writing the manuscript. MZM: contributed to data collection, contributed to analysis of the data, interpretation of data, and writing the manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Flow chart of patients' selection.

Figure S2. Hazard ratios (95% confidence intervals) of the association between resistin (per 10 ng/ml increase) and death with a functioning graft (panel a), all-cause mortality (panel b) and death censored graft loss (panel c) using multivariable adjusted Cox regression analyses in 988 kidney transplant recipients in various subgroups of patients (*P*-values are the significance levels of the interaction terms).

Table S1. Correlation between serum resistin level and different variables.

Table S2. Number observed events and mortality rates for death with functioning graft, all-cause mortality and graft loss divided by resistin tertiles.

Table S3. Association between resistin (per 10 ng/ml increments) and outcomes in 988 kidney transplant recipients.

Table S4. Association between different resistin tertiles and outcomes in 988 kidney transplant recipients.

Table S5. Association of serum resistin with graft-loss censored mortality in 988 kidney transplant recipients estimated with Fine-Gray competing-risks analysis, where death with a functioning graft was the event of interest and graft failure before death was considered as a competing event.

Table S6. Number of missing values of the variables used for adjusted Cox regression models.

Table S7. STROBE Guideline.

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